85

87-024752/04 B04 D16 WAKU- 04.06.85
WAKUNAGA SEIYAKU KK *J6 1280-292-A
04.06.85-JP-121249 (10.12.86) C12n-15 C12p-21
Protein prepn. by exo-bacterium secretion - involves host transformation by introducing recombined DNA into host bacterium cell
C87-010334

A new method for the prepn. of a protein by an extracellular bacterial secretion comprises:
(A) constructing a vector contg. the promoter originated from an alkaline phosphatase gene and a gene coding the signal sequence under the control of this gene, and which can replicate in bacterium host cell;
(B) a gene coding the foreign protein is integrated in to this vector and the recombinant DNA is used to transform the bacterium host cell;
(C) transformed cells are cultured in a medium contg. inorg. phosphorus in amt. insufficient for the induction of protein synthesis and sufficient for the growth of bacteria, and then transferred to a medium to which inorg. phosphorus or a medium contg. it is added at a constant rate; and
(D) the foreign protein is recovered from the cultured liq.

B(4-B4A5) D(5-C12) / B0114

USE/ADVANTAGE
The protein is obtd. by a simple genetic engineering method.

EXAMPLE
The vector used is pTA 1529 (I) which is prepd. from pTA 529 and pHS 1.
A gene coding humun-epithelial cell growth factor (II) is combined with (I) to give recombinant DNA (III). E. coli K 12 YK 537 is transformed by (III) to give transformed cells (IV).
(IV) is cultured in LB medium and then in M-9 medium to give a liquid which is then passed through a Prep PAK column and then a DEAE-TOYOPEARL column to collect the desired fraction (II). (18ppW97LDDwgNo0/1).

J61280294-A

87-024753/04 B01 D16 HONS 06.06.85
YAKULT HONSHA KK *J6 1280-293-A
06.06.85-JP-121488 (10.12.86) C12p-33 C12r-01/64
Steroid phosphoric acid ester prepn. - by microbial conversion using Mortierello fungus
C87-010335

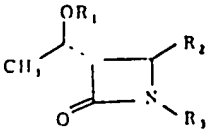
The process includes a step in which a filamentous fungus belonging to Mortierella species, and able to phosphatise a steroid cpd., is contacted with a steroid cpd. or its alkaline metal salt. Subsequently, the phosphate of the steroid cpd. is recovered.
USE - Prepn. of highly water-sol. steroid cpd. In an example, 6 l liq. medium contg. 50 g glucose, 5 g peptone, 2 g yeast extract, 1 g KH_2PO_4, 2 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 10 mg $CaCl_2$, 10 mg $FeSO_4.7H_2O$, 10 mg thiamine-HCl, 1 g taurolithocholic acid and 1 l water is fed into a 10 l fermentor and Y 2-1 species previously cultured in the same medium annas above at 27 deg.C for 48 hrs. is inoculated into the medium and cultured at 27 deg.C for 5 days with irring at 300 rpm and aeration of 0.5 vvm (pH: 7-7.5). Then, the cultured liq. is cooled at 50 deg.C and centrifuged to give a clear supernatant liquor. It is passed through an Amberlite XAD-2 column and the absorbed bed is eluated by methanol. The eluate is mixed with an extract of the centrifuged solid and concentrated in vacuo and absorbed on a Sephadex LH20 column and it is eluted by chloroform/methanol and then eluated by methanol, and the latter

B(1-D1) D(5-C4) / B0115

water-sol. fraction is conc. in vacuo to give 3.5 g solid. It is purified by a DEAE-Sephadex A-25 column and a XAD-2 column to give 2.1 g of Na taurolith ocholic acid 3-phosphate. (7pp Dwg.No.0/0)

87-024754/04 B03 D16 SANY 06.06.85
SANKYO KK *J6 1280-295-A
06.06.85-JP-121479 (10.12.86) C12p-41 C12r-01/01
Optically active hydroxyethyl azetidinone derivs. prepn. - from optically inactive acyloxyethyl azetidinone derivs. using microorganisms or enzymes
C87-010336

Optically active β-lactam cpds. (I; R_1=H) are produced by selective hydrolysis of racemic cpds. of formula (I) using a microorganism or an enzyme.



(I)

R_1 = opt. substd. acyl;
R_2 = opt. substd. alkyl, alkenyl, alkynyl, aryl, alkylthio, alkylsulphonyl, arylthio or arylsulphonyl or acyloxy; and
R_3 = H or protective gp. for N atom.

USE/ADVANTAGE
Optically active 3-(1-hydroxyethyl)-2-azetidinone deriv.

B(7-D1) D(5-C) / B0116

is obtd. from optically inactive 3-(1-acyloxyethyl)-2-azetidinone deriv.
These optically active azetidinone derivs. are important intermediates for carbapenem and penem derivs. which have antibacterial activity.

MICROORGANISM
This may be chosen from bacteria, yeast and fungi:
Bacteria:
Arthrobacter simplex SANK 73560 (IAM 1660);
Chromobacterium violaceum SANK 72783 (ATCC 31532);
Flavobacterium capsulatum SANK 70979 (IFO 12533);
Flavobacterium meningosepticum SANK 70779 (IFO 12535); or
Bacillus subtilis SANK 76759 (IAM 1069);
Yeast:
Aureobacidium pullulans SANK 10877 (ATCC 15232);
Candida albicans SANK 50169 (IFO 0683);
Pichia farinosa SANK 58062 (IAM 4003);
Pichia terricola SANK 51684 (FERM 8001);
Rhodotorula minuta SANK 50871 (IFO 0932); or
Saccharomyces cerevisiae SANK 50161 (IAM 4512);
Fungi:

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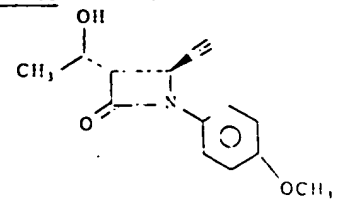
Aspergillus niger SANK 13658 (ATCC 9142);
Gliocladium roseum SANK 10560 (FERM 8259); or
Humicola asteroidea SANK 14981 (FERM 8260).

ENZYME

This may be of microorganism or animal or plant cell origin, examples of which are:
esterase (carboxylic-ester hydrolase, EC 3.1.1.1, e.g. pig liver originated commercial prod. PLE);
lipase (triacylglycerol acylhydrolase, EC 3.1.1.3, e.g. Aspergillus oryzae or Aspergillus niger-originated commercial prod.);
aminoacylase (N-Amino acid aminohydrolase, EC 3.5.1.14 e.g. commercial prod. prepd. from Aspergillus genus of fungi).

Commercially available low-cost crude prod. such as Takadiastase (originated from Aspergillus oryzae) contains lipase and may be used in place of purified standard lipase.

EXAMPLE



dl-3,4-Trans-1-(4-methoxyphenyl)-3-[(1R*)-1-acetoxyethyl]-4-ethynyl-2-azetidinone (60 mg) was subjected to shaken culture with Pichia farinosa SANK 58062 (IAM 4303) at 30°C for 24 hrs.

Culture liquor was extracted with ethyl acetate, and obtd. crude prod. (76 mg) was purified by silica gel TLC (cyclohexane/ethyl acetate = 1/1, U.V. lamp detection, Rf = 0.32) to give (21 mg) of (3S,4S)-1-)4-Methoxyphenyl)-3-((1R)-1-hydroxyethyl)-4-ethynyl-2-azetidinone. $(\alpha)_D^{24}$ = -135° (C=1, $CHCl_3$). (22ppW-69LDDwgNo.0/0).

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87-024755/04 B05 D16 SUMO 05.06.85
SUMITOMO CHEM IND KK *J6 1280-296-A
05.06.85-JP-121944 *(10.12.86)* C12p-41
Biochemical prepn. of optically active phenoxy phenoxy propanol - involves reacting bacterial esterase with opt. sald. organic carboxylic acid ester
C87-010337

B(10-E4B) D(5-A2C) I
B0117

Optical biochemical resolution of (+-) -2-(4-phenoxyphenoxy) propene-1-ol (I) comprises interacting esterase produced by microorganism selected from the gp. consisting of Pseudomonas, Chromobacterium, Arthrobacter, Alcaligenes, Candida, Achromobacter, Nocardia, Flavobacterium, Toluiopsis, Brevibacterium, Bacillus, Escherichia, Micrococcus, Hansenula, Mucor, Corynebacterium, Mycobacterium, Saccharomyces, Thermomyces, Humicola, Thizopus, Aspergillus, Streptomyces, Geotricum, Treoderma, Acinetobacter, Aeromonas, Beauveria, Rhodotorula, Enterobacter, Penicillium, Serratia, Erwinia, Staphylococcus, Phycomyces, Propionibacterium, Metarrhizium, Paeciiomyces, Saccharomycopsis, Verticillium and X anthomonas, with organic 1-18C opt. sald. carboxylic acid. ester of (+-)-(I) to resolve to optically active (I) and its antipode ester.

Cultivation is conducted at 20-40 deg.C for 1-3 days in liq. medium. As esterase there are used culture liquid, cells sepd. from the culture liq., crude esterase sepd. from the cells or culture filtrate, culture filtrate contg. esterase, purified esterase and esterase-contg. extract of concentrate. Reaction is conducted under shaking or stirring. The reaction temp. is 10-70 deg.C. To keep the pH constant during the reaction, buffer such as sodium phosphate and sodium acetate can be used. Use concn. of the substrate is 0.5-80 wt.%, pref. 10-50 wt.%. Pref. 2-12C organic carboxylic acid is used.

ADVANTAGE - Process gives optically active (I) with very high optical purity. (11pp Dwg.No.0/0)

87-024756/04 B04 D16 S03 (D13) NODA 04.06.85
NODA INST SCI RES *J6 1280-297-A
04.06.85-JP-119782 *(10.12.86)* C12q-01/26 G01n-33/50
Determn. of amadori cpd. in e.g. soy sauce - by treatment with fructosyl:amino acid oxidase and e.g. determn. of hydrogen peroxide
C87-010338

B(4-B2C2, 4-B4B, 4-B4D5, 4-D1, 7-A2, 10-B2J, 11-C7B1, 12-K4A) D(5-A2A) S
B0118

Determination of Amadori cpd. comprises treating a liq. contg. Amadori cpd. with fructosylamino acid oxidase in the presence of oxygen, and determg. the amt. of oxygen consumed in the oxidn. reaction or determg. hydrogen peroxide formed by the reaction.

Reagent for the determn. of Amadori cpd. contains fructosylamino acid oxidase.

Amadori cpd. is that formed from aldose and alpha-amino acid, namely fructosylalanine from glucose and alanine or hydroxyacetonylglycine from glyceraldehyde and glycine. Sample liq. contg. Amadori cpd. is e.g. soy sauce, honey, etc. Fructosylamino acid oxidase used is pref. that obtd. by cultivating microorganism, esp. bacteria belonging to Corynebacterium genus (e.g. Corynebacterium sp. No.2-3-1). The determn. of oxygen is carried out by oxygen electrode, and that of hydrogen peroxide by colorimetry.

ADVANTAGE - The determn. of Amadori cpd. can be easily carried out. Amadori cpd. reflects the state of food (e.g. soy sauce) or infusion liq. during mfr. or storage. Amadori cpd. bound by protein can be determd. after conversion into its free state by the reaction with a suitable peptidase. This is useful for the examination of diabetes mellitus. (8pp Dwg.No.0/0)